PENETRATION, DAMAGE, AND REPRODUCTION OF *Meloidogyne arenaria* ON PEANUT

By

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This dissertation is dedicated to the agriculture in Panama which was the inspiring force that gave me the impetus to succeed in this difficult, but thrilling learning experience. I came to the University of Florida to learn as much as I could with the purpose of serving our farmers in the best way I know--through nematological research aimed at finding solutions to practical problems. I only hope that the experience and ideas gained from this work will help in developing a better agriculture in my beloved country.

With deep appreciation and admiration to my former professor and advisor Dr. Alejandro Ayala, nematologist at the University of Puerto Rico, Mayaguez Campus, who introduced me to the science of Nematology. He gave me all the support that I needed and inspired me to continue learning.

This work is also dedicated to my mother Olivia Maria, my wife Olga Lydia, and our children Jesica Eileen and Elvia Lineth. It is my hope that this effort will inspire my children in the future to pursue noble causes no matter how difficult they may look.

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Abstract of Dissertation Presented to the Graduate School
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Field microplot experiments demonstrated that initial population densities of *Meloidogyne arenaria* race 1 as low as 2 nematodes (a mixture of eggs and juveniles)/100 cm^3 of soil caused yield reduction of peanut cv. 'Florunner'. The percentage of galled pods was shown to be an important criterion to consider in terms of quality and marketability of the product. The possibility of using linear regressions for predicting yields of peanut in fields infested with the nematode was shown also. However, in order to attain good predictability, several seasons of research are needed to obtain regression lines that include most of the variations that occur from season to season.

Under greenhouse conditions penetration of peanut roots by *M. arenaria* race 1 (population FL-157) occurred as soon as 12 hours after inoculation. Vegetative growth of peanut was not affected by the parasite, but the reproductive growth stages were affected as measured by the number of pegs, the number of nongalled pods, the fresh and dry weights of nongalled pods, and the percentage of galled pods.

Fresh weight of nuts was affected significantly by M. arenaria when both the roots and the pegs were infected. Dry weight of nuts was affected significantly when only the roots or when both roots and pegs were infected by the nematode. Infection of the roots only caused a dry weight loss of 17 %, infection of the pegs only caused a dry weight loss of 4 %, and infection of both roots and pegs caused a loss of 17.5 %.

Populations FL-183, FL-202, and NC-204 of M. arenaria race 2 did not penetrate the roots of 'Florigiant', 'Florunner', or 'Valencia' peanut under varied greenhouse and growth chamber conditions.

CHAPTER I
INTRODUCTION

The peanut, Arachis hypogaea L., is the second most important edible legume grown worldwide. It originated in South America and is grown in about 80 countries on all six continents (Minton, 1984). During the 1979-80 growing season an estimated 17.4 metric tons of peanuts were produced on 18.2 million hectares (Anonymous, 1981). About 65 % of the total world production is grown in India, the Peoples' Republic of China, the United States, Senegal, and the Sudan. Peanut ranks ninth in acreage among the most important row crops in the United States and is second in dollar value per acre (Minton, 1984).

Nematodes constitute an important limiting factor in peanut production. The estimated percenage loss of peanuts due to nematodes in the United States between 1962 and 1968 was calculated at 10 % (Anonymous, 1971). On this basis, the total loss due to nematodes in the United States during 1979 was approximately 200.8 million kilograms with a value of $91.5 million (Minton, 1984).

The major plant parasitic nematodes associated with peanut are species of Belonolaimus Steiner, 1949; Criconemella de Grisse and Loof, 1965, Luc and Raski, 1981; Meloidogyne Goeldi, 1887; and Pratylenchus Filipjev, 1936.

Of these, Meloidogyne arenaria (Neal, 1889) Chitwood, 1949, and M. hapla Chitwood, 1949, are considered to be the most important economic pests (Porter et al., 1982). Meloidogyne javanica (Treub, 1885) Chitwood, 1949, also was reported feeding on peanut (Martin, 1961; Minton et al., 1969). Only M. arenaria has been reported infecting peanut in Florida (Dickson, 1985).

Yield losses of peanut caused by M. arenaria, the peanut root-knot nematode, may exceed 50 % in heavily infested fields, but since the nematode usually is distributed randomly instead of uniformly, average yield losses may be less than 50 % (Minton, 1984). Although much effort has been expended to find resistance or tolerance in peanut to M. arenaria, none has been found (Miller, 1972; Minton and Hammons, 1975; Holbrook et al., 1983). Consequently, the most practical and effective way for managing this nematode and improving quantity and quality of peanuts is through the use of crop rotation and nematicides (Porter et al., 1982). However, to avoid the overuse of nematicides and the subsubsequent higher production costs, possible environmental pollution, lower rate of return per dollar invested, and human health implications, it is important to know the nature of damage and economic threshold levels of M. arenaria on peanut (Porter et al., 1982).

There are few reports on the assessment of yield losses caused by _M_. _arenaria_ on peanut in the United States (Rodriguez-Kabana et al., 1982). Most of these reports are related to the evaluation of different nematicides for the management of this parasite (Dickson and Smart, 1971; Rodriguez-Kabana and King, 1979; Rodriguez-Kabana et al., 1980; Minton and Bell, 1981; Rodriguez-Kabana et al., 1981; Minton et al., 1982; Rodriguez-Kabana et al., 1982a; Rodriguez-Kabana et al., 1982b; Dunn, 1983). Studies to explain the relationships between the initial populations of nematodes and the yields for predictive purposes are scarce (Seinhorst, 1965; Oostenbrink, 1966). Field microplots provide the opportunity to manipulate nematode populations and to determine their approximate damage threshold levels under relatively natural conditions. Threshold levels obtained in this manner can be validated later under natural field conditions and adjusted if necessary (Duncan and Ferris, 1983).

Understanding the host-parasite relationships is important to the selection of the most effective and economical management strategies for production. However, research has been limited in the past mainly to the peanut crop itself, the nematode and its management, and its pathological effects on the crop. Pathological relationships that occur when _M_. _arenaria_ race 1 penetrates and reproduces on different vegetative and reproductive growth stages of peanut are unknown. For example, it is not known whether nematode infection of the roots or of the pegs and pods has a greater influence on the total yield loss of peanut, or whether infection of roots may predispose pegs and pods to infection.

Although there are two races of _M_. _arenaria_, only race 1 has been reported infecting peanut (Taylor and Sasser, 1978; Osman et al., 1985). These races are morphologically and cytologically identical (Sasser and Cameron, 1982; Cliff and Hirschmann, 1985; Osman et al., 1985; Triantaphyllou, 1982). It would be of interest to determine if the soil environment can be manipulated or the physiology of the peanut plant may be altered in such a way as to induce race 2 to infect peanut. There are reports of differences in pathogenicity within species and even within populations of a race (Minton, 1963; Kirby et al., 1975).

The major objectives of these investigations were to determine 1) the damage threshold level of _M_. _arenaria_ race 1 on peanut, cv. 'Florunner'; 2) the effects of _M_. _arenaria_ race 1 on the vegetative and reproductive growth of peanut; 3) the percentage of the total yield loss caused by infection of the roots, of the pegs and pods, and of infection to both pegs and pods and roots; and 4) whether _M_. _arenaria_ race 2 can be induced to infect peanut.

CHAPTER II
DAMAGE THRESHOLD LEVEL OF *Meloidogyne arenaria* RACE 1 ON PEANUT CULTIVAR 'FLORUNNER'

Introduction

The assessment of peanut yield losses caused by the peanut root-knot nematode is limited to the work done by Rodriguez-Kabana et al., 1982. They grouped data from sixteen field experiments over a period of three years comparing the yields from naturally infested plots to yields from nematicide-treated plots. Their results indicate that significant peanut yield losses were caused by this parasite. Populations of second stage juveniles in the soil at or near harvest were used to determine the relationship between yield and population density of the nematode. Quadratic equations described the association although linearity was not rejected. In contradiction to Seinhorst's (1982) findings, these equations do not support the hypothesis that there is a minimum population density of juveniles in the soil at which no yield reduction occurs. Seinhorst's conclusions were drawn from field experiments with the potato cyst nematode, *Globodera rostochiensis* (Wollenweber), 1927, and were used to explain these relationships in many different crops and nematode species under many different conditions.

Jones (1965), Barker et al. (1979), and Greco et al. (1982) were among the first to use field microplots in the assessment of crop damage by nematodes. Their value in studies of host-parasite relationships has been demonstrated (Barker and Olthof, 1976; Olthof, 1978), but their use is not widespread because they are expensive, and installation is difficult requiring special equipment (Barker et al., 1979; Johnson et al., 1981).

Materials and Methods

A field microplot experiment was established in 1983 and repeated in 1984 to study the relationship between the initial population density of *M. arenaria* race 1 and the yield and quality of peanut, cv. 'Florunner'.

A completely randomized design with 10 replicates per treatment was used in both experiments. In 1983, the initial population densities were a mixture of 0, 2, 10, 50, 150, and 250 nematodes (a mixture of eggs and juveniles) /100 cm^3 of soil. The second year the levels evaluated were 0, 2, 10, 30, 50, 100, and 150 nematodes (a mixture of eggs and juveniles) /100 cm^3 of soil.

The experiments were conducted in 76-cm diameter microplots enclosed with 60-cm cylinders of flat fiberglass inserted 50-cm into the soil (Johnson et al., 1981). The cylinders were arranged in rows 1.5 m apart in Arredondo fine sand (93.2 % sand, 4.3 % silt, 2.5 % clay, 1.5 % organic matter, and pH 5.8) treated with 960 kg/ha of methyl bromide applied under a 1 mil plastic cover 2 months before planting. A 4-8-16 fertilizer was incorporated into the soil at the rate of 660 kg/ha. No plant-parasitic nematodes were detected when the microplots were sampled before planting.

Eggs and juveniles of M. arenaria race 1 were extracted with 0.5 % NaOCl (Hussey and Barker, 1973) from the roots of infected tomato (Lycopersicon esculentum Mill. 'Rutgers') grown in a greenhouse. The inoculum in a water suspension was taken to the field immediately after extraction and sprinkled uniformly over the entire surface area of each microplot which had been prepunched with 54 uniformly spaced holes divided evenly to provide depths of 7, 14, and 23 cm. Only water was added to the controls. Each microplot was raked to a depth of 7-10 cm immediately after infestation, starting with the control to avoid contamination.

Peanut cv. 'Florunner' seeds were soaked in a suspension of Nitragin^R at the rate of 498 g/1 of water/120 kg of seed. Five pairs of seeds were placed in holes 5-cm deep dug at the vertices of a pentagonal pattern (Fig. 1). After germination, the plants were reduced to five per microplot. A 90-cm tall chicken-wire fence was placed around each plot to prevent the plants from inserting pegs into the soil outside the plot area (Fig. 2). Gypsum (calcium sulfate) was sprinkled around each plant at blooming at the rate of 660 kg/ha.

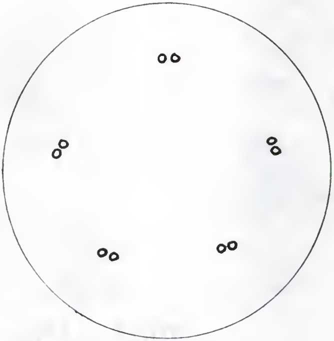

Fig. 1. Pentagonal pattern used for planting, with two planting holes at each vertex. Plants were thinned to one per vertex after germination.

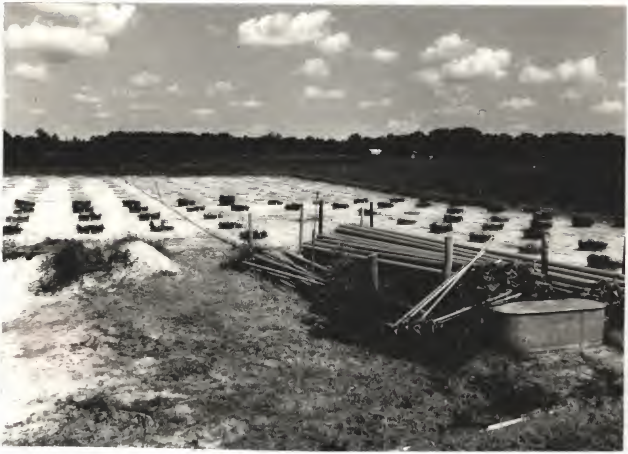

Fig. 2. Microplots used in the 1983 and 1984 damage threshold experiments. Agronomy Farm.

Acephate (Orthene^R) at the rate of 2.5 ml/l of water and chlorothalonil (Bravo 500 F^R) at the rate of 2 ml/l of water were mixed together in a 19-liter back sprayer and sprayed initially every 2 weeks for a period of 6 weeks and then weekly or twice a week, as needed, to control insects and leafspot, Cercospora arachidicola Hori, respectively. Weeds were controlled by pulling them from within the microplots and by hoeing as needed in between microplots.

The soil moisture was monitored constantly with a rain gauge and the plots were irrigated to field capacity when necessary. Nematode sampling began one month after planting and continued at 30-day intervals until harvest. One sample, composed of 5 cores 20-cm deep through the root zone of each plant, was taken from each microplot using a stainless steel sampling cone with an opening of 2.5-cm (Esser et al., 1965).

Nematodes were extracted from 100 cm^3 of soil using a centrifugal-flotation technique (Jenkins, 1964). Second-stage juveniles and males were counted directly under a dissecting scope.

The experiment was terminated when the plants reached maturity. Beginning with the control plots, all plants were dug with a shovel to expose the pods and left in the microplots for 24 hours. Then pods were pulled from the pegs and the percentage of infected pods was estimated by separating galled from nongalled pods. The pods were dried for two days in a hot air dryer set at 60 C. The moisture content of the seeds was determined and the sample from each plot weighed. Yields were standardized at a 14 % moisture content (Young et al., 1982) in both tests. Regression equations were used to determine the relationship between the initial nematode population densities in the soil and the yield. All statistical analyses were done using the version 3.0 of the MSTAT computer program package from the University of Michigan. The percentages of infected pods were first transformed, using the arcsine transformation, prior to the statistical analysis (Rohlf and Sokal, 1981). Duncan's Multiple Range Test was used to separate the treatment means (Duncan, 1955).

Results

In 1983, yields of peanuts were reduced significantly when the initial population of the nematode was 150 nematodes (a mixture of eggs and juveniles)/100 cm^3 of soil. At this level, yield was reduced by about 100 g compared to the control (Table 1).

Pegs and pods on the control plants were free of galls. The percentage of galled pods was 26.5 % when the initial population of the nematode was 2 nematodes/100 cm^3 of soil. However, this latter treatment was statistically inferior from the rest in which the percentage of galled pods ranged from 62 to 86 % (Table 1, Figs. 3 and 4).

Table 1. Effects of the initial population density of Meloidogyne arenaria race 1 on the percentage of galled pods and the yield of peanut cv. 'Florunner' during 1983.

Nematodes/ 100 cm^3 of soil	% galled[1] pods	Weight of pods (g)[1]	Yield loss(g)
0	0.0 a[2]	250.25 a	-
2	26.5 b	220.45 a	29.80
10	62.0 c	230.75 a	19.50
50	74.9 cd	210.40 a	39.85
150	84.2 cd	150.00 b	100.25
250	86.0 d	117.00 b	133.25

[1] p=0.01

[2] Mean of 10 observations. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test.

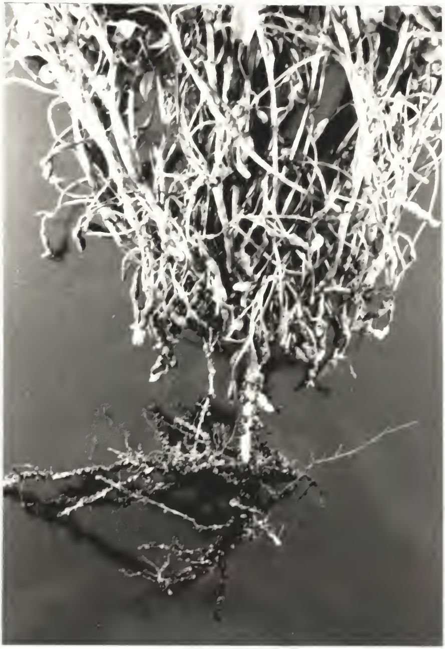

Fig. 3. Harvested plant showing many heavily galled pegs that were unable to form pods.

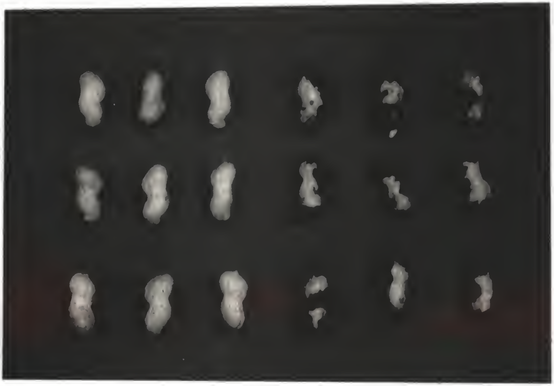

Fig. 4. Nongalled pods from the control plants (left) and galled pods from infected plants (right).

Second stage juveniles and males of M. arenaria were first detected in soil samples 60 and 101 days after planting, respectively. The population of juveniles increased steadily from the second month after planting to harvest. Population densities of juveniles were particularly high during September and October. Ratios of Pf/Pi ranged from 45 to 1761 and were inversely proportional to the initial population (Table 2).

The relationship between the initial population of the nematode and the yield was linear. The linear regression used to describe this association showed that even an initial population of 2 nematodes/100 cm^3 of soil was negatively correlated with yields (Fig. 5).

Significant differences occurred between the yield in the control plots and the yields in plots of each of the inoculum levels evaluated during 1984 (Table 3). The yield obtained with 2 nematodes/100 cm^3 of soil in 1984 was comparable to that obtained from the control plots in 1983 (Table 2). Yields from the other treatments in 1984 were extremely low compared to those obtained in 1983.

The percentages of galled pods were all significantly higher than the control in which no galled pods were found. In general, the percentages of galled pods were higher in 1984 than in the previous year.

Juveniles were detected 58 days after planting and their numbers increased throughout the sampling period.

Table 2. The number of second-stage juveniles and males of *Meloidogyne arenaria* race 1 in microplots planted with peanut cv. 'Florunner' during 1983.

Nematodes/ 100 cm^3 of soil	Juveniles and males/100 cm^3 of soil[1]					Pf/Pi
	Aug 10	Sept 20		Oct 20		
	J-2	J-2	Male	J-2	Male	
0	0	0	0	0	0	-
2	16	289	11	3523	24	1761
10	4	1183	14	3884	65	388
50	16	3604	64	9445	98	189
150	16	5842	132	16030	169	107
250	20	3482	38	11299	101	45

[1] Means of 10 replicates.

[2] Pi=initial population; Pf=final population.

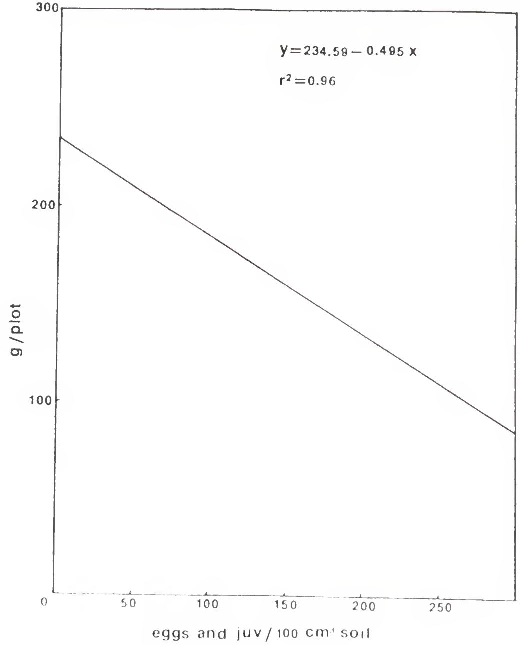

Fig. 5. Effect of the initial population density of Meloidogyne arenaria race 1 on the yield of peanut cv. 'Florunner' under field microplot conditions. 1983.

Table 3. Effects of the initial population density of *Meloidogyne arenaria* race 1 on the percentage of galled pods and the yield of peanut cv. 'Florunner' during 1984.

Nematodes/ 100 cm^3 of soil	% galled pods	Yield/plot (g)	Yield loss (g)
0	0.0 a[1]	493.61 a	-
2	49.0 b	305.03 b	188.58
10	62.0 bc	152.71 c	340.90
30	69.0 c	104.34 cd	389.27
50	70.5 c	65.56 d	428.05
100	93.5 d	42.61 d	451.00
150	95.0 d	40.03 d	453.58

[1] Mean of 10 observations. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.01).

Males were first detected 88 days after planting. Ratios of Pf/Pi were higher in 1984 (Table 3) than in 1983 and were inversely proportional to the initial populations of the nematode (Table 4).

The relationship between the yield and the initial population of the nematode was not linear. The power curve ($Y=aX^b$) explained better the relation between these two variables (Fig. 6). This curve was linearized to obtain a regression line for predictive purposes. The resulting equation is $Y'=2.72-0.52(\log X)$, with an r^2 (determination coefficient) of 0.99, and significant regression and correlation coefficients (Fig. 7).

Discussion

The yield and quality of peanut cv. 'Florunner' are affected severely by M. arenaria race 1. The marketable yield depends upon the initial population of the nematode at planting because the damage caused to peanut by the peanut root-knot nematode is a combined effect of weight reduction, low quality of pods due to galling, and abortion of pegs. Even though many infected pegs never produce pods, they serve as reproduction sites for the parasite becoming heavily galled with many egg masses present.

During 1983, the damage threshold level of the nematode was an initial population density of more than 50/100 cm^3 of soil. Significant yield reductions began at a

Table 4. The number of second-stage juveniles and males of Meloidogyne arenaria race 1 in microplots planted with peanut cv. 'Florunner' during 1984.

Nematodes/ 100 cm^3 of soil	Juveniles/100 cm^3 of soil[1]							Pf/Pi
	Jun 6	Jul 6		Aug 13		Aug 28		
	J-2	J-2	Male	J-2	Male	J-2	Male	
0	-	-	-	-	-	-	-	-
2	22	33	0	5412	144	8078	40	4039
10	77	170	0	3421	140	8009	75	801
30	130	299	0	4611	139	5417	14	181
50	155	328	2	4271	63	5300	27	106
100	558	405	9	3283	47	3395	16	34
150	379	626	16	3701	38	3992	6	27

[1] Means of 10 replicates.

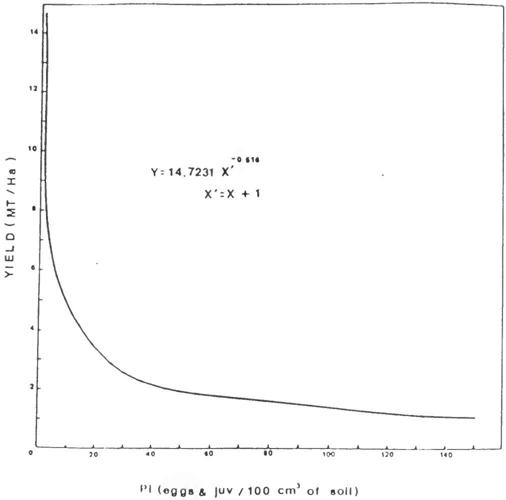

Fig. 6. Yield response of peanut cv. 'Florunner' to different initial population densities of Meloidogyne arenaria race 1 under field microplot conditions. 1984.

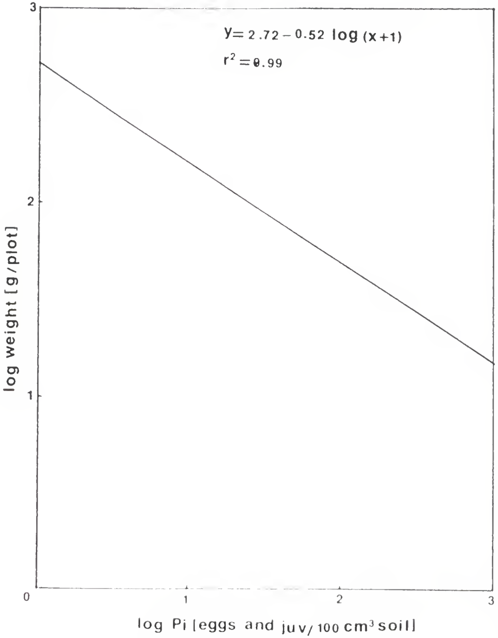

Fig. 7. Effect of the initial population density of *Meloidogyne arenaria* race 1 on the yield of peanut cv. 'Florunner' under field microplot conditions. 1984.

population level of 150 nematodes/100 cm^3 of soil. Since there were no inoculum levels between 50 and 150, it is possible that the damage threshold level was lower than 150. This assumption agrees with the results of Rodriguez-Kabana et al. (1982) who found that in naturally infested soils yield losses caused by M. arenaria may occur at populations lower than 50 juveniles/100 cm^3 of soil. The linear relationship between the initial population of the nematode and the yield of 'Florunner' peanut in the 1983 experiment was in part caused by the large intervals between inoculum levels.

The percentage of infected pods in 1983 proved to be an important criterion in evaluating damage because the yield from the control as measured by weight did not differ statistically from that of the lower inoculum levels; however over 25 % of the pods at the lower inoculum levels were galled which lowered the grade and marketable value.

Rate of nematode reproduction was inversely proportional to the initial population which is in agreement with the widely accepted criteria that crowding and availability of food have an impact on reproduction, development and sex expression. It is during such stress periods that males appear in the soil at low to moderate population densities (Triantaphyllou, 1982).

In 1984, the damage threshold level was around 2 nematodes/100 cm^3 of soil, but the yield was comparable, or even superior to the control in 1983.

The percentage of galled pods in 1984 also proved to be an important factor in evaluating damage since about 50 % of the pods in the 2-nematodes/100-cm^3-of-soil treatment were galled by the nematode. Some of these pods were so badly damaged that they would have been discarded, and market value of those less galled would have been lowered. In those treatments with the higher two inoculum rates, the proportion of infected pods was so high (>93 %) and the yield so low that the marketable product was negligible.

Yields and the initial populations of the nematode were not linearly related which probably was the result of the treatments (nematode rates) being at such close intervals. Relatively small changes in the initial population densities of the nematode caused large variations in yields. After linearization, the resulting line showed a highly significant regression coefficient and a high coefficient of determination (0.99) which indicates that this is a good regression line under the experimental conditions. The reproduction rate of the nematode changed from one growing season to the other indicating that climatic conditions may have influenced this ratio.

The regression lines obtained from these experiments can be used to predict peanut yields in fields infested with

M. arenaria race 1. However, regression lines obtained from microplots should be validated under field conditions before practical use is made of them, because seasonal effects may cause changes in the reproduction of the nematode (Rodriguez-Kabana et al., 1982).

The fact that significant yield losses occurred with low population densities of the nematode suggests that there is always the need to manage this parasite.

CHAPTER III

EFFECTS OF *Meloidogyne arenaria* RACE 1 ON THE VEGETATIVE AND REPRODUCTIVE GROWTH STAGES OF PEANUT cv. 'FLORUNNER'

Introduction

In order to better understand the damage that *M. arenaria* causes to peanut it is important to have information about the effects of the nematode on the different growth stages of the crop. However, there is a lack of such information.

Growth of the peanut plant is divided into vegetative and reproductive stages (Boote, 1982). Vegetative growth may continue throughout much of the life of the plant, but can be divided into stages with each node on the main stem being one stage beginning with the cotyledonary node. The reproductive phase is considered to have up to nine different stages associated with flowering, pegging, fruit growth, seed growth, and maturity. Vegetative and reproductive stages and the various reproductive stages may overlap during plant development.

A series of experiments was started in August, 1985, to study the penetration of *M. arenaria* race 1 into the roots and its effects on the vegetative and five reproductive growth stages of peanut cv. 'Florunner'.

Materials and Methods

Meloidogyne arenaria was collected originally from peanut near Williston, Florida, and reared on tomato cv. 'Rutgers' in a greenhouse.

Root Penetration

This experiment was designed to determine when do infective juveniles begin penetration and the percentages of these juveniles that entered peanut roots, at different time intervals after inoculation, over a 96 hr period. Seeds of peanut were germinated and the seedlings transplanted into plastic centrifuge tubes containing 50 cm^3 of steam-sterilized soil. The seedlings were allowed to grow for two weeks before the nematode inoculum was added (Fig. 8).

Freshly hatched juveniles were obtained by placing several egg masses in 50-ml plastic tubes half-filled with distilled water and bubbling air through the water constantly. Juveniles collected during the first 24 hr were discarded. Juveniles collected during the next 24 hr were used as inoculum. The peanut seedlings were inoculated with about 50 juveniles per tube and maintained in an air-conditioned greenhouse with a mean temperature of 25 C (range 15-32 C). Three plants were harvested 6, 12, 24, 48, 72, and 96 hours after inoculation. Roots were washed carefully from the soil, cleared and stained (Byrd et al., 1983) in

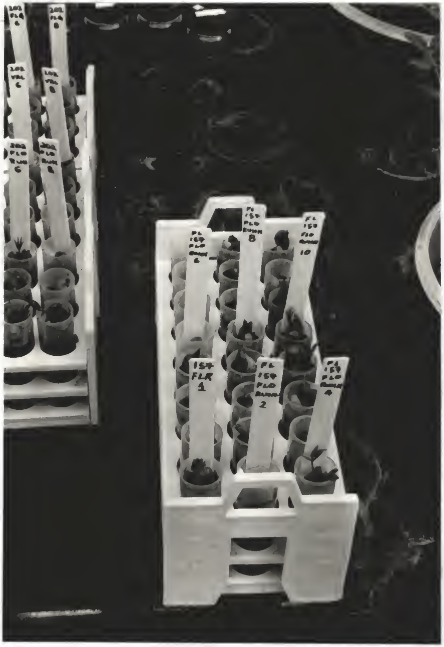

Fig. 8. 'Florunner' peanut seedlings growing in plastic tubes containing 50 cm^3 of steam-sterilized soil. 50 second-stage juveniles of Meloidogyne arenaria race 1 were inoculated two weeks after transplant.

order to determine the number of juveniles inside. Juveniles remaining in the soil were removed by centrifugation-flotation and counted.

Vegetative Growth Stages Experiment

A second experiment was designed to determine the effects of four population densities of *M. arenaria* on top and root growth of peanut and the number of nodes produced by the plants over a 54-day period of vegetative growth. Treatments were 0, 10, 50, and 100 nematodes (eggs and juveniles)/100 cm^3 of soil and were replicated 5 times. The experimental units were arranged in a completely randomized design. Germinated 'Florunner' seeds, previously soaked in a suspension of Nitragin^R, were transplanted one each in the center of a 25-cm diameter pot containing 7,500 cm^3 of steam-sterilized soil. Seven days after transplanting the seedlings were inoculated as described above. The plants were maintained in a fiberglass-covered shadehouse with screened walls and a mean temperature of 30 C (range 25-35 C). The plants were sprayed every 7-14 days with Orthene^R, Kelthane^R or both at the rates of 5 and 2.5 ml per liter of water, respectively, to control arthropod pests (Fig. 9).

Evaluations were made 54 days after planting when the plants were starting to set pods. The number of nodes on the main stem, fresh and dry weights (hot air dried at 60 C for 2 days) of tops and roots, and the egg mass index were

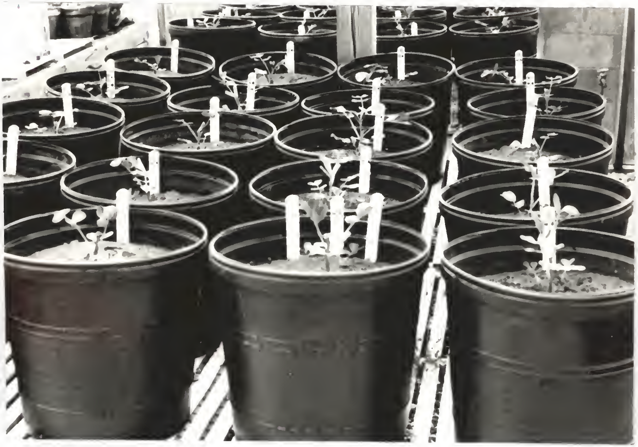

Fig. 9. Plastic pots containing 7,500 cm^3 of steam-sterilized soil used to study the effects of Meloidogyne arenaria race 1 on the vegetative and reproductive growth stages of peanut cv. 'Florunner'.

recorded. The index was based on the following scale proposed by Taylor and Sasser (1978): 0=0 egg masses; 1=1-2 egg masses; 2=3-10 egg masses; 3=11-30 egg masses; 4=31-100 egg masses; 5=101 or more egg masses.

Reproductive Growth Stages Experiments

Three experiments were designed to determine the effects of M. arenaria on five reproductive growth stages: beginning bloom (R1), beginning peg (R2), beginning pod (R3), full pod (R4), and harvest maturity (R8). Inoculum levels tested were mixtures of 0, 10, 50, and 100 eggs and juveniles/100 cm^3 of soil. A completely randomized design with 5 replicates per treatment was used in all the experiments. Inoculations were made at the same time in all the experiments.

Evaluations of the effects of M. arenaria on the R1 and R2 stages came from one set of 20 plants and included the number of days to begin bloom, number of days to begin peg, and number of pegs per treatment. Evaluations on the effects on R3 and R4 stages came from a second set of 20 plants and included the effects of M. arenaria on the number of days to begin pod, number of full pods per treatment, number of galled and nongalled pods per treatment, fresh and dry weight of pods, fresh and dry weight of nongalled pods, mean weight per pod, and galling and egg mass indices on the roots. The mean number of eggs per egg mass also was determined by selecting 2 root systems at random from each of the treatments and counting the eggs in 5 egg masses from each root system. The gelatinous matrices containing the eggs were dissolved in a 10 % NaOCl solution (Tyler, 1938) to separate the eggs for counting. All parameters, except the beginning of pods (R3), were measured at the end of the full pod stage (R4), 62 days after planting.

Evaluations of the effects of M. arenaria on the R8 (harvest maturity) stage came from a third set of 20 plants and included the percentage of infected pods, the fresh and dry weight of pods, the dry weight of seeds alone, and the shelling percentage. This latter parameter is defined as the percentage of the total dry weight accounted for by the seed. Insect control and drying of plant materials were as described for the vegetative growth experiment.

Results

Root Penetration

Juveniles were not found in the roots prior to 24 hr after inoculation at which time about 52 % were inside the roots (Table 5). Their numbers increased slowly up to 96 hr when 92 % of the juveniles were inside the roots. All juveniles that remained in the soil were still active.

Vegetative Growth Stages Experiment

There were no statistical differences between treatments in the number of nodes on the main stem or in the fresh and dry weights of the tops 54 days after planting but root weights and egg mass indices were significantly

Table 5. Penetration by second-stage juveniles of _Meloidogyne_ _arenaria_ race 1 into roots of peanut cv. 'Florunner' in early vegetative growth stage.

Hrs after[1] inoculation	Juveniles[2] remaining in soil	Juveniles[2] inside the roots	Percentage of penetration
6	47	0	--
12	46	0	--
24	23	25	52.1
48	13	31	70.4
72	12	35	74.5
96	4	45	92.0

[1] Inoculation with 50 freshly-hatched juveniles.

[2] Mean of 3 replicates.

Table 6. Effects of three densities of _Meloidogyne arenaria_ race 1 on the number of nodes on the main stem, the fresh and dry weights of tops and roots, and the egg-mass index during the vegetative growth of peanut cv. 'Florunner', 54 days after planting.

Nematodes/ 100 cm^3 of soil	Number of nodes on main stem	Weight of tops (g)		Weight of roots (g)		Egg mass index[2]
		Fresh	Dry[1]	Fresh	Dry[1]	
0	14.20	62.91	12.34	18.56 a[3]	2.28 a	0.00 a
10	13.20	75.68	16.76	35.27 b	4.95 b	3.60 b
50	13.40	72.84	15.44	28.49 ab	4.30 ab	4.00 b
100	13.00	72.44	14.57	27.96 ab	3.57 ab	5.00 c

[1] Dry weight at 60 C for 2 days.

[2] 0=0 egg masses; 1=1-2 egg masses; 2=3-10 egg masses; 3=11-30 egg masses; 4=31-100 egg masses; 5=101 or more egg masses (Taylor and Sasser, 1978).

[3] Means of 5 replicates. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.05).

different with the control treatment having the lowest weight (Table 6). No statistical differences were observed between the control and either the 50 or 100 inoculum rates. However, at the 10 inoculum rate both fresh and dry weights were significantly greater than the controls. No galls were observed on the roots throughout this 54-day period of growth, but egg masses were found in all treatments except the control. The egg-mass index varied from 0 for the control to 5 at the 100 inoculum level.

Reproductive Growth Stages Experiments

Meloidogyne arenaria did not show any adverse effects on the number of days to the onset of blooming (R1), pegging (R2), pod set (R3), or the number of full pods (R4) (Table 7). The number of pegs formed was reduced significantly from the control only at the 100 inoculum level.

The number of nongalled pods in the controls was significantly greater than in the 100 inoculum level treatment, but not greater than in the 10 and 50 inoculum level treatments. The number of galled pods was significantly higher in all the treatments that received nematodes than in the control (Table 8). The percentage of galled pods ranged from 41 % at the 10 inoculum level to 59 % at the 100 inoculum level. Total yield, expressed as fresh and dry weights of full pods (galled + nongalled), was not reduced by M. arenaria race 1 by the end of the R4 stage (full pods). However, the fresh and dry weights of nongalled pods were

Table 7. Effects of *Meloidogyne arenaria* race 1 on different reproductive growth stages (R1, R2, R3, and R4) of peanut cv. 'Florunner'.

Nematodes/ 100 cm^3 of soil	Days to begin blooming (R1)	Days to begin pegging (R2)	No. of pegs	Days to pod set (R3)	No. of full pods (R4)
0	25.4	31.0	51.4 a[1]	41.6	11.6
10	26.6	33.4	44.2 ab	44.0	14.2
50	25.0	32.8	44.0 ab	43.2	14.6
100	25.2	35.0	26.6 b	46.0	9.8

[1] Mean of 5 replicates. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test ($p=0.05$).

Table 8. Effects of *Meloidogyne arenaria* race 1 on the number of nongalled and galled pods and the fresh and dry weights of pods during the R4 stage of peanut cv. 'Florunner', 62 days after planting.

Nematodes/ 100 cm^3 of soil	Nongalled pods	Galled pods	% pods galled	Total weight of pods (g)		Wt of nongalled pods(g)	
				Fresh	Dry[1]	Fresh	Dry[1]
0	11.6 a[2]	0.0 a[2]	-	28.71	6.92	28.71 a[2]	6.92 a[2]
10	8.4 ab	5.8 b	41	32.92	6.70	19.46 ab	3.93 ab
50	8.0 ab	6.6 b	45	33.55	6.91	18.44 ab	3.74 ab
100	4.0 b	5.8 b	59	20.76	4.04	8.78 b	1.79 b

[1] Dry weight at 60 C for 2 days.

[2] Mean of 5 replicates. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.01).

significantly less than the control at the 100 inoculum level. At this level the dry weight of the nongalled pods was 74 % less than in the control, whereas the fresh weight was 69 % less.

The peanut root-knot nematode did not cause any reduction of the fresh and dry weights of individual pods at R4 under the experimental conditions (Table 9). No galls or egg masses were observed on the roots of the control plants. At the 10 inoculum level the galling index was significantly less than that at the 50 and 100 inoculum levels. Egg mass indices at the 10 and 50 inoculum levels were significantly less than at the 100 inoculum level. The number of eggs per egg mass was significantly higher at the 10 inoculum level than at the other two inoculum levels (Table 9), but the product of the egg-mass index times the number of eggs per egg mass was very similar indicating that total egg production was about the same at all inoculum levels.

There were highly significant differences in the percentages of infected pods when the data were subjected to an angular transformation prior to the statistical analysis (Table 10). However, no differences were detected between treatments in the fresh and dry weights of pods, dry weights of seeds or the shelling percentages at harvest maturity.

Table 9. Effects of different population densities of *Meloidogyne arenaria* race 1 on the weight per pod, galling and egg-mass indices, and number of eggs per egg mass at the R4 reproductive stage of peanut cv. 'Florunner', 62 days after planting.

Nematodes/ 100 cm^3 of soil	Wt per pod(g)		Infection indices[2]		No. of[3] eggs per egg mass
	Fresh	Dry[1]	Galling	Egg mass	
0	2.46	0.58	0.0 a[4]	0.0 a	--
10	2.31	0.46	3.8 b	3.2 b	1080.0 a
50	2.31	0.48	4.8 c	3.8 b	772.5 b
100	2.09	0.36	5.0 c	5.0 c	630.0 b

[1] Dry weight at 60 C for 2 days.

[2] 0=0 egg masses; 1=1-2 egg masses; 2=3-10 egg masses; 3=11-30 egg masses; 4=31-100 egg masses; 5=101 or more egg masses (Taylor and Sasser, 1978).

[3] Means of 10 egg masses.

[4] Means of 5 replicates. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.01).

Table 10. Effects of different population densities of *Meloidogyne arenaria* race 1 on the percentage of galled pods, the fresh and dry weights of pods, the dry weight of the seeds, and the shelling percentage of peanut cv. 'Florunner' at harvest maturity (R8).

Nematodes/ 100 cm^3 of soil	Galled pods (%)		Wt of pods (g)		Dry weight[2]	Shelling percentage[3]	
	Observed	Transformed[1]	Fresh	Dry[2]	of seeds (g)	Observed	Transformed[1]
0	0.0	0.00 a[4]	64.03	34.42	27.90	81.06	64.14
10	46.8	42.10 b	81.41	40.54	31.52	77.75	61.61
50	65.0	56.93 bc	84.40	39.13	29.90	76.41	59.37
100	86.0	73.15 c	82.71	38.24	29.31	76.65	60.24

[1] Observed percentages were angularly transformed using the arcsine transformation prior to the statistical analysis.

[2] Dry weight at 60 C for 2 days.

[3] The proportion of the total dry weight of pods accounted for by the seed.

[4] Mean of 5 replicates. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.01).

Discussion

Root Penetration

The fact that no juveniles were found inside the roots 12 hr after inoculation indicates that the nematodes needed at least this period of time to locate a root, find a suitable penetration site, and enter the root. This elapsed time could have been affected by the population density, the proximity to the roots as well as the nonnatural condition imposed by handling. Juveniles were first observed inside the roots 24 hr after inoculation. Thus penetration and entry took place between 12 and 24 hr after inoculation for some of the nematodes, but since others took up to 96 hr to enter the roots they may not have been as near a suitable penetration site as those that entered earlier.

Vegetative Growth Stages Experiment

The peanut root-knot nematode, _M_. _arenaria_ race 1, did not have any detrimental effects on the number of nodes on the main stem or the fresh and dry weights of tops, 54 days after planting. The fact that both fresh and dry weights of roots were less for the control treatment than for the 10 inoculum level indicates that the nematode had a stimulatory effect on root growth that probably was manifested in the galled tissue but may have been due to a production of more roots due to infection by the nematode. A stimulating effect on plant growth caused by different nematode species has been reported by others (Coursen and Jenkins, 1958; Apt and Koike, 1962; Malek and Jenkins, 1964). The growth of peanut, among other crops, has been reported to be stimulated by various species of Meloidogyne, including M. arenaria (Madamba et al., 1965). According to these authors, stimulation of plant growth occurred primarily at low infection levels of the nematode.

The fact that no root galls were observed after 54 days of plant growth seems odd in view of the fact that egg masses were present in all the treatments in which nematodes were inoculated, indicating that reproduction occurred normally. Perhaps development of the original population of the nematode (inoculated juveniles) is slow on peanut and galling occurs only after the first generation of juveniles invade the roots and start feeding.

Reproductive Growth Stages Experiments

The infection of the roots by M. arenaria race 1 did not have any significant effect on the R1 and R2 reproductive stages. However, the number of pegs at the beginning of R3 was significantly reduced at the 100 inoculum level. This could indicate that infection by the nematode alters the reproductive mechanism of the plant in some manner that could be related to the greater root weight produced during the vegetative growth stages. Perhaps, as the plants produced either more or heavier roots to compensate for the damage caused by the nematode, less nutrients were partitioned to the tops and hence pegging physiology was altered.

Although there were no significant differences between treatments in the number of full pods produced when the plants reached the R5 stage (beginning seeds), the numbers of galled and nongalled pods were statistically different. Quality of the pods was superior for the controls since all the pods were free of galls. For the rest of the treatments, quality of the pods, as measured by galling, was reduced significantly by approximately the same amount. For peanut, both yield and quality help determine the price and marketability of the product. Although total yield was not statistically reduced by the presence of the nematode, the yield of nongalled, high quality pods was affected by high initial population densities of the parasite. However, the lack of statistical differences between treatments in terms of total yield may be attributed to the fact that the plants were growing in a relatively small container which may have prevented healthier plants from maximum production. The shadehouse protected environment in which temperature, humidity, weed, insect, and disease control were optimal, may have allowed infected plants to better tolerate infection by the nematode. Since shadehouse and greenhouse experimental conditions do not simulate field conditions, it may be advisable to use field microplots or field plots when more precise assessments of yield losses are needed.

By the end of the R4 stage, galls and egg masses were noticeable on all inoculated plants, and reproduction by the nematode was high indicating that the females were developing normally. However, since development of the nematode seemed slow during early growth stages of the plant, perhaps addition of a nematicide at the beginning of pegging would protect the pegs and pods from nematode damage and prevent build-up of the nematode populations. The efficacy of nematicides can be significantly improved if their applications are properly timed.

Yield, as measured by weight, and shelling percentage of 'Florunner' peanut at harvest maturity (R8) were not affected by the peanut root-knot nematode. The reasons for the failure in detecting differences in yield between the treatments may be similar to those expressed for the R4 stage, but undoubtedly quality and market value of galled pods were lowered, so that monetary value was less even though weight was not.

CHAPTER IV

YIELD LOSSES OF PEANUTS CAUSED BY *Meloidogyne arenaria* race 1: A COMBINED EFFECT OF LOSSES DUE TO DAMAGE TO THE ROOT SYSTEM AND TO THE PEGS AND PODS

Introduction

Yield loss of peanut caused by the peanut root-knot nematode is due both to reduced yield and reduced quality due to the unsightly appearance of pods galled by the nematode. Such pods can not be sold for use as unshelled peanuts and their value is lowered when sold for other uses. Therefore, damage to peanuts by this nematode is very significant because roots, pegs, and pods may be infected up until harvest. However, the percentage of damage caused by root infection and the percentage caused by infection of pegs and pods are not known. Nor is it known whether infection of the roots predisposes the pegs and pods to infection or increases the severity of damage.

These experiments were designed to determine yield losses of peanut due to infection of roots only, of pegs and pods only, and of roots, pegs and pods.

Materials and Methods

The experimental unit used to contain roots separated from pegs and pods consisted of an 8,000 cm^3 clear plastic pan and a PVC^R pipe with a length of 41 cm having an internal diameter of 5.12 cm. A 5.62-cm hole was bored in the bottom center of the pan and the PVC pipe inserted through it, positioned 2.5 cm below the rim of the pan, and sealed to the pan with PVC cement. The lower end of the pipe was sealed with a circular piece of laminated plastic with a small drain hole in its center (Fig. 10). The pan and the pipe were filled with 6,600 cm^3 and 600 cm^3 of steam-sterilized soil, respectively. Soil levels in the pan and pipe were 1.5 cm below their respective rims to prevent nematodes in either the pan or the pipe from contaminating the other area. A piece of black cardboard was taped around each pan to keep the soil from excessive sunlight in the pegging area. To facilitate uniformly watering the soil in the PVC pipe holes 2 cm apart were made along the sides of a 25 cm long clear, soft plastic tube having a 5 mm internal diameter. One end of the tube was plugged with cotton and slipped into the soil inside the PVC pipe to a depth of 20 cm. Water was added as needed by injecting it into the tube with a hypodermic needle. A previously germinated seed that had been soaked in a suspension of Nitragin^R was planted in the center of the PVC pipe and allowed to grow for one week prior to inoculation (Fig. 11). Then 100 nematodes (a mixture of eggs and juveniles)/100 cm^3 of soil extracted previously from 'Rutgers' tomato were added (Hussey and Barker, 1973).

The treatments were noninoculated roots, noninoculated pegs (NIR/NIP); noninoculated roots, inoculated pegs

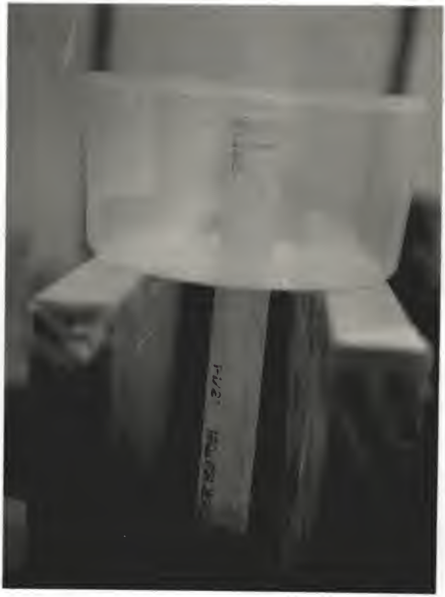

Fig. 10. Experimental unit designed to allow separate inoculation of roots and pegs. Roots were grown in the PVC^R pipe (with 600 cm^3 of steam-sterilized soil) and pegs were set in the pan (with 6,600 cm^3 of steam-sterilized soil).

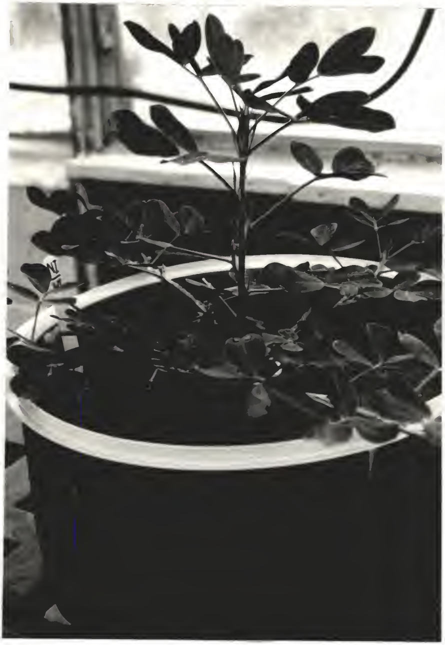

Fig. 11. Control plant of peanut cv. 'Florunner' growing under greenhouse conditions at a mean temperature of 25 C. The sides of the pan were covered to avoid excessive sunlight.

(NIR/IP); inoculated roots, noninoculated pegs (IR/NIP); and inoculated roots, inoculated pegs (IR/IP). A completely randomized design with 4 replicates per treatment was used. The plants were grown in an air-conditioned greenhouse with a mean temperature of 25 C (range 15-32 C). When the plants formed pegs, the pegs entered the soil contained in the pan. This was facilitated by the relatively small diameter of the PVC pipe, and the few pegs that formed over the PVC pipe were manually reoriented into the pan before they penetrated the soil (Fig. 12).

Water was added as needed to the soil in the pans with a mist tip connected to the hose to avoid splashing. Fertilization, insect control, and disease control were done as described for Chapter III.

The soil in the pans was inoculated with 100 nematodes/100 cm^3 of soil when 50 % of the plants reached the R3 stage (beginning pods) and had at least one elongated peg with the ovary tip beginning to enlarge to at least twice the peg diameter (Boote, 1982). The plants were evaluated 135 days after planting when they reached harvest maturity.

Fresh and dry weights of pods, number of full pods, number of infected pods, and the galling index per treatment were determined. Dry weight of pods, galling index, and all statistical analyses were measured as described in Chapter III.

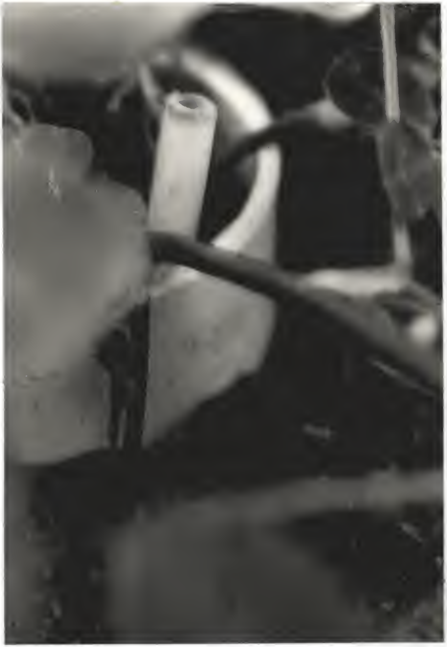

Fig. 12. Plant setting pegs out of the root zone.

Results

There were significant differences between the treatments in terms of the fresh and dry weights of the pods (Table 11). The fresh weight of pods in the control treatment was significantly different only from the pods in the treatment in which both the roots and the pegs were inoculated. Consequently, the percentage of fresh weight reduction was not significant when only the roots or only the pegs were inoculated. When both roots and pegs received nematodes, a significant reduction of about 22 % in the fresh weight of pods occurred, compared to the control.

In terms of dry weight of the pods, no differences were observed between the treatments with noninoculated roots, i. e. the control and the treatment in which only pegs were inoculated (Table 11). However, these two treatments showed significant differences from those in which the roots were inoculated. The percentage reduction of the dry weight of pods was significantly less when pegs only were inoculated than when the roots only or both roots and pegs were inoculated. About 17 % of the total yield in terms of dry weight was lost due to the infection of roots only and of both roots and pegs. The infection of the pegs (and later the pods) alone did not cause significant reductions in the dry weight of the pods.

Significant differences were observed in the number of full pods only between the control and the treatment in

Table 11. Effects of the separate and combined infection of the roots and the pegs of 'Florunner' peanut by *Meloidogyne arenaria* race 1 on the percentage reduction in fresh and dry weights of pods.

Treatments[1]	Description[2]	Fresh weight (g)	Weight loss (g)	% of weight loss	Dry[3] weight (g)	Weight loss (g)	% of weight loss
IR/NIP	Noninoculated	17.70 a[4]	--	--	8.73 a	--	--
NIR/IP	Pegs only inoculated	17.08 a	0.62	3.50	8.40 a	0.33	3.78
IR/NIP	Roots only inoculated	15.18 ab	2.52	14.24	7.25 b	1.48	16.95
IR/IP	Both inoculated	13.72 b	3.98	22.49	7.20 b	1.53	17.53

[1] NIR/NIP=noninoculated roots, noninoculated pegs; NIR/IP=noninoculated roots, inoculated pegs; IR/NIP=inoculated roots, noninoculated pegs; IR/IP=inoculated roots, inoculated pegs.

[2] The inoculum consisted of a mixture of 100 eggs and juveniles/100 cm^3 of soil.

[3] Dry weight for 2 days at 60 C.

[4] Means of 4 replicates. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.05).

Table 12. Effects of the separate and combined infection of the roots and the pegs of 'Florunner' peanut by *Meloidogyne arenaria* race 1 on the number of full pods, the number of galled pods, the percentage of galled pods, and the root-galling index.

Treatments[1]	Number of full pods	Galled pods		Root-galling index
		Number	%	
NIR/NIP	11.75 a[2]	0.00	0	0
NIR/IP	10.25 ab	10.25	100	0
IR/NIP	10.25 ab	0.00	0	5
IR/IP	8.25 b	8.25	100	5

[1] NIR/NIP=noninoculated roots, noninoculated pegs; NIR/IP=noninoculated roots, inoculated pegs; IR/NIP=inoculated roots, noninoculated pegs; IR/IP=inoculated roots, inoculated pegs.

[2] Means of 4 observations. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (p=0.05).

which both roots and pegs were inoculated (Table 12). The infection of the roots alone or of the pegs and pods alone did not cause a significant reduction in the number of full pods at harvest maturity. Quality was again an important difference between treatments which is not reflected in the statistical analysis. Whereas clean quality pods were observed for the control and the roots-only-inoculated treatments, 100 % of the pods were infected in both treatments in which the pegs were inoculated. However, the infection was judged of medium severity when only the pegs were inoculated, receiving a gall rating of 2 on a 0-5 scale (Taylor and Sasser, 1978) compared to the both-roots-and-pegs-inoculated treatment in which all the pods were rated 5. Galling indices of the roots were 5 in those treatments in which the roots were inoculated. No galls were found on the roots of any other treatments.

Discussion

Damage only to the roots was not enough for *M. arenaria* race 1 to cause a significant reduction of the yield of peanut in terms of fresh weight of pods. Significant losses of about 22 % in the fresh weight of pods occurred only when both the roots and the pegs were infected by the parasite. This was probably the result of the significant reduction in the number of full pods. It seems to indicate that an alteration of the physiology of the plants occurred either by reducing the pegging ability of the plants and/or making the pegs more susceptible to damage when the roots were first infected by the parasite. The fact that both the fresh weight of pods and the number of full pods were not significantly different from the control when only the pegs were inoculated corroborates this.

Dry weight of the pods was a more sensitive indicator than was fresh weight in showing differences in yield of peanut pods due to the peanut root-knot nematode since significant differences from the control were found even when only the roots were inoculated. Excess of water in the tissues could have been responsible for the lack of significance in the fresh weight of pods when only the roots were inoculated.

For both the fresh and dry weights, damage to the roots contributed the most to the total yield reduction.

CHAPTER V
INFECTIVITY AND REPRODUCTION OF *Meloidogyne arenaria* RACE 2 ON PEANUT

Introduction

Although there are two host races of *M. arenaria*, only *M. arenaria* race 1 has been reported infecting peanut (Taylor and Sasser, 1978; Osman et al., 1985). These two host races are morphologically identical. It is not possible to separate them by cytological studies since the triploid cytological race A of *M. arenaria* may include both physiological races 1 and 2 (Sasser and Cameron, 1982; Triantaphyllou, 1982) Although race 2 may be found together with race 1 sharing the same soil ecosystem (Kirby et al., 1975), it has never been reported infecting peanut under field conditions. Diomande et al., 1981, reported the penetration in roots of peanut cv. 'NC 3033' by two race 2 populations (256 and 486) under greenhouse conditions. Although both populations were reported to enter peanut roots, only population 256 reproduced slightly.

Under stress conditions (lack of suitable hosts, soil temperature, etc.) nematodes may develop "mutants" or "pathotypes" capable of breaking plant resistance to their infection. This is especially true of apomictic species which may undergo these changes due to their genetic plasticity (Triantaphyllou, 1982). There are reports of differences in pathogenicity within species and even within populations of a race of the genus *Meloidogyne* (Minton, 1963; Kirby et al., 1975).

Soil temperature is known to affect the rate of metabolism and development of nematodes (Tyler, 1933; Bergeson, 1959; Ferris et al., 1978). Temperature can be measured in terms of the number of cumulative degree days above 10 C, considered the basal threshold temperature for development of species within the genus *Meloidogyne* (Tyler, 1933). Since soil temperature may also affect plants in a similar way (Dropkin, 1969; Ferris, 1976), this parameter seems to be useful in studies of host-parasite interactions.

Changes in the physiology of plants may occur when their roots are infected by nematodes. These changes may predispose roots so that they may become infected by species or races that normally are not parasitic on them. The split-root technique has proven to be useful in studies of this kind (Hinson, 1975; Mc Intyre and Miller, 1976; Eisenback, 1983; Ko et al., 1984). A loss of resistance to *Meloidogyne incognita* was reported in tobacco when *M. arenaria* infected one-half of the root system (Eisenback, 1983).

Does *M. arenaria* race 2 penetrate the roots of peanut under normal soil temperature and moisture conditions? Can the soil environment be manipulated or the physiology of the peanut plant altered in such a way as to induce race 2 to infect and reproduce on peanut? Is it possible to induce race 2 to reproduce on peanut by allowing undeveloped eggs to be in contact with the roots for long periods of time? A penetration study, a soil temperature study, a split-root experiment, and a time study were designed to answer these questions.

Materials and Methods

Root Penetration

The penetration of three populations of *M. arenaria* race 2 on three peanut cultivars, 'Florigiant', 'Florunner', and 'Valencia', was evaluated in an air-conditioned greenhouse with a mean temperature of 25 C (range 15-32 C). The nematode populations under study were FL-183 and FL-202, from Florida, and NC-204, from North Carolina. Population FL-183 was collected originally at Suwannee County from 'G-28' tobacco (*Nicotiana tabacum* L.), and population FL-202 was collected in Holmes County from 'Davis' soybean (*Glycine max* (L.) Merr.). Population NC-204 originated in North Carolina from 'NC-95' tobacco.

Freshly hatched juveniles of all three populations were used as inoculum. The juveniles were collected from egg masses detached from the roots of 'Rutgers' tomato and placed inside an extraction chamber using a 16 m Nitex^{R} screen. All juveniles recovered 24 hours after placing the egg masses in the chamber were discarded. Juveniles that hatched during the next 24 hours were used as inoculum to insure uniformity in age.

Previously germinated seeds of all three cultivars were planted in plastic centrifuge tubes containing 50 cm^3 of steam-sterilized soil. The seedlings were allowed to grow for two weeks before inoculation. All inoculations were made immediately after the second collection of juveniles. Fifty second-stage juveniles of *M. arenaria* were added to each tube. Three replicates of each nematode population and of each peanut cultivar were harvested at 6, 12, 24, 48, 72, and 96 hours after inoculation. After clearing and staining the roots (Byrd et al., 1983), the juveniles inside the entire root systems were counted. Juveniles remaining in the soil were extracted using a centrifugal-flotation technique (Jenkins, 1964) and counted.

Soil Temperature Experiments

Four different constant soil temperatures were evaluated for their temperature effects on the penetration of peanut cultivars by three populations of *M. arenaria* race 2. Using the procedures described by Tyler (1933), the optimum constant temperature for reproduction of *Meloidogyne arenaria* over a period of 31 days was calculated to be 28 C.

Previously germinated seeds of each of the cultivars were planted in plastic pots containing 1,000 cm^3 of steam-sterilized soil. The pots were placed in two temperature-controlled tanks, one maintained at 20 C and one at 24 C.

After the completion of this test in 31 days, the temperatures in the tanks were set at 28 and 34 C, respectively, and another test conducted at those temperatures. The temperature-controlled tanks were located inside a growth room illuminated by fluorescent lamps for 14 hours daily. The plants were allowed to grow for one week before inoculation with 50 nematodes (a mixture of eggs and juveniles)/ 100 cm^3 of soil. Plants were harvested 31 days after inoculation. Treatments were replicated 4 times and a control 'Rutgers' tomato plant was included for each of them to check the viability of the inocula (Fig. 13). Evaluations were made by washing the roots carefully and staining them with phloxine B (Fenner, 1962; Holbrook et al., 1983) to facilitate detection and counting of the egg masses.

Split-root Experiment

When the hypocotyls of the peanut seeds were about 2 cm in length, they were split with a sterile razor blade up to the cotyledon, held apart with a sterile piece of wood and placed back into a petri dish for 3 days to allow callus tissue to form. Then the seedlings were transplanted into 10-cm diameter clay pots filled with steam-sterilized soil to allow root growth. A week later the soil was rinsed carefully from the roots and the two halves planted in separate 10-cm diameter clay pots filled with steam-sterilized soil (Fig. 14). A week later one half of the root system in each of the 11 replicates received 8,000 nematodes

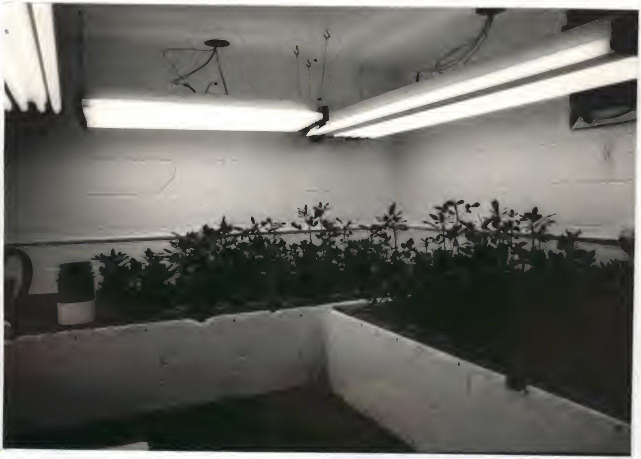

Fig. 13. Three peanut cultivars ('Florigiant', 'Florunner', and 'Valencia') inoculated with three populations of Meloidogyne arenaria race 2 (FL-183, FL-202, and NC-204) growing in temperature-controlled water tanks at two different constant temperatures (28 and 34 C) for 31 days.

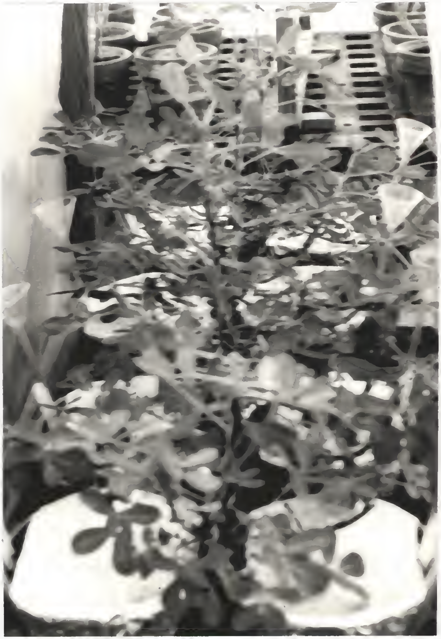

Fig. 14. Split-root technique. Peanut cv. 'Florunner' first inoculated with Meloidogyne arenaria race 1 on one half of the root system. The other half received M. arenaria race 2 after 21 days from first inoculation.

(a mixture of eggs and juveniles) of M. arenaria race 1 population FL-157, and 21 days later the other half of the root system received 8,000 M. arenaria race 2 population FL-202 (Eisenback, 1983). Five 'Rutgers' tomato plants were also inoculated with the race 2 population to check inoculum viability. All plants were maintained in an air-conditioned greenhouse with a mean temperature of 25 C (range 15-32 C). After 45 days from the second inoculation, the roots were evaluated for galling and production of egg masses after carefully washing and staining the roots with phloxine B.

Time Study

Previously germinated seeds of 'Florunner' peanut were planted in clay pots containing 4,000 cm^3 of steam-sterilized soil. The seedlings were allowed to grow for one week in an air-conditioned greenhouse at a mean temperature of 25 C (range 15-32 C) and then were inoculated with one of three populations (FL-183, FL-202, and NC-204) of M. arenaria race 2.

A single inoculum rate of 40,000 nematodes (a mixture of eggs and juveniles composed mostly of undeveloped eggs collected from new egg masses produced by the first generation of young reproductive females) per pot (10/cm^3 of soil) was used for each of the three race 2 populations of the nematode (Hussey and Barker, 1973). There were 4 replicates per treatment, and a control 'Rutgers' tomato plant was included for each of the treatments to check the viability of the inocula.

Evaluations were made by washing and staining the roots with phloxine B at 135, 155 or 175 days. The 'Rutgers' tomato plant was evaluated in the same way 45 days after inoculation.

Results

Root Penetration

The juveniles of the three race 2 populations studied did not enter the roots of any of the three peanut cultivars (Table 13), with two exceptions: one juvenile of population NC-204 was found in one replicate of 'Florunner' peanut 24 hours after inoculation, and two juveniles of population FL-183 were found in one replicate of 'Valencia' peanut 24 hours after inoculation. Active juveniles were recovered from the soil in all the samples showing that the nematodes were still alive but did not penetrate the root.

Soil Temperature Experiment

After 31 days under constant temperatures, no reproduction of race 2 was observed in any of the treatments. However, all the 'Rutgers' tomato plant roots had an egg mass index of five.

Split-root Experiment

The egg-mass index was five in all the root halves that received race 1 FL-157, but no reproduction was observed in any of the root halves that received race 2 FL-202 (Fig. 15). The roots of all the 'Rutgers' tomato plants inoculated with race 2 were heavily galled having an egg

Table 13. Number of juveniles of three populations of *Meloidogyne arenaria* race 2 remaining in the soil and inside the roots of three peanut cultivars.

Cultivar/ race 2 population[1,2]	Time after inoculation (hours)											
	6		12		24		48		72		96	
	S[3]	R	S	R	S	R	S	R	S	R	S	R
cv. 'Flg'												
FL-183	46[4]	0	44	0	46	0	42	0	42	0	45	0
FL-202	43	0	44	0	44	0	48	0	47	0	46	0
NC-204	41	0	45	0	42	0	42	0	42	0	44	0
cv. 'Flr'												
FL-183	47	0	47	0	47	0	47	0	43	0	42	0
FL-202	44	0	47	0	40	0	49	0	45	0	45	0
NC-204	48	0	46	0	46	.3	46	0	44	0	49	0
cv. 'Val'												
FL-183	42	0	46	0	46	.6	47	0	47	0	49	0
FL-202	48	0	48	0	47	0	47	0	45	0	45	0
NC-204	46	0	44	0	44	0	44	0	45	0	46	0

[1] cv. 'Flg'='Florigiant'; cv. 'Flr'='Florunner'; cv. 'Val'='Valencia'.

[2] Original sources in Florida of two field populations of *M. arenaria* were Suwannee County, for FL-183--'G-28' tobacco (*Nicotiana tabacum* L.); Holmes County, for FL-202--'Davis' soybean (*Glycine max* (L.) Merr.). The original source in North Carolina for NC-204--'NC-95' tobacco (*N. tabacum* L.).

[3] S=soil; R=root.

[4] Means of three replicates.

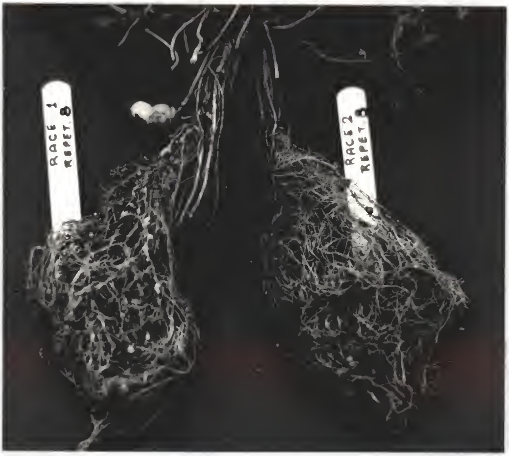

Fig. 15. One half of the root system of peanut cv. 'Florunner' galled by Meloidogyne arenaria race 1 (left). The other half inoculated with M. arenaria race 2 is free of galls (right).

mass index of 5 meaning that the inoculum was viable but unable to reproduce on peanuts.

Time Study

No reproduction of the race 2 FL-202 was found in any of the three peanut cultivars under study in any of the three time periods during which the nematodes were left in contact with the roots. All 'Rutgers' tomato plants roots were heavily galled and had egg-mass indices of 5 showing that the inoculum was viable.

Discussion

Under normal conditions of soil moisture and temperature, populations FL-183, FL-202, and NC-204 of *M. arenaria* race 2 do not penetrate the roots of 'Florigiant', 'Florunner', or 'Valencia' peanut cultivars. The detection of one and two juveniles inside the roots of 'Florigiant' and 'Valencia' cultivars, respectively, is strongly suspected to be the result of a contamination with race 1, since this and the race 1 penetration study were done simultaneously. Penetration occurred within the same period it was reported to commence for race 1 (see Chapter III).

Exposing the nematodes and the plants to soil temperatures that are optimum or either below or above the optimum for the reproduction of the nematode did not have any effect on the infectivity of the nematode on peanut roots or in the ability of the crop to resist race 2 infection. The fact that all the 'Rutgers' tomato controls were heavily galled and had egg masses indicates that the inoculum was viable but not parasitic on peanut.

Changing the physiology of the peanut plant by a heavy infection of its roots with race 1 of the peanut root-knot nematode does not impair its ability to resist infection by race 2.

Leaving the roots of peanut exposed to the inoculum of race 2 for long periods of time did not cause any changes in the plants or in the nematodes to promote infection and reproduction. 'Florigiant', 'Florunner', and 'Valencia' peanut cultivars appear to be immune to *M. arenaria* race 2, populations FL-183, FL-202, and NC-204.

CHAPTER VI
CONCLUSIONS

The results of field microplot experiments indicate that yield and quality of 'Florunner' peanut can be severely affected by the peanut root-knot nematode. Even low preplant populations of the nematode may reduce yields significantly although a seasonal effect was observed between the two growing seasons. The mean damage threshold level was 76 nematodes (a mixture of eggs and juveniles)/100 cm^3 of soil for both seasons, but more data are needed to get a better estimate. This indicates the necessity of repeating these experiments for several growing seasons to obtain a mean regression line which includes most of the variations from season to season. This predictive line should then be adjusted to natural field conditions.

Juveniles of _M_. _arenaria_ race 1 population FL-157 penetrated and entered the roots of 'Florunner' peanut between 12 and 24 hours after inoculation, under greenhouse conditions.

In the shadehouse, the vegetative stages of 'Florunner' peanut were not affected by the presence of the nematode except for a greater root weight when 10 nematodes (a mixture of eggs and juveniles)/100 cm^3 of soil were inoculated. The onset of the various reproductive stages was not affected, but some plant responses such as number of pegs, number of galled and nongalled pods, and weight of nongalled pods were affected particularly at the initial population of 100 nematodes/100 cm^3 of soil. The weight per seed and the shelling percentage at harvest maturity were not reduced by the nematode.

Under greenhouse conditions the reduction in the fresh weight of pods was significantly increased over the control when both roots and pegs were inoculated and the effect on the fresh weight loss was synergistic compared to the sum of the individual effects. On the other hand, the effect of the combined infection to the roots and pegs on the dry weight loss was additive. Dry weight was more sensitive than fresh weight in detecting yield differences between treatments. Up to 97 % of the total dry weight reduction in peanut due to _M_. _arenaria_ race 1 is attributed to the infection of the roots alone. Dry weight reductions due to infection of the pegs alone were up to 22 %.

Under greenhouse and growth chamber conditions, 'Florigiant', 'Florunner', and 'Valencia' peanut cultivars are nonhosts to _M_. _arenaria_ race 2 populations FL-183, FL-202, and NC-204.

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BIOGRAPHICAL SKETCH

Eric M. Candanedo Lay was born to Cesar Candanedo and Olivia M. Lay on November 13, 1948, in Panama, Republic of Panama. His elementary school education was completed in the Escuela Republica de Argentina in 1960. He graduated from high school in the Instituto Nacional de Panama in 1966. After high school he attended the University of Puerto Rico, Mayaguez Campus, from 1969 to 1976 obtaining his B. S. and M. S. in agriculture. For his master's thesis he worked with chemical control of nematodes parasitic on pineapple under the supervision of Dr. Alejandro Ayala. Since 1977, he has been working in nematological research at the Instituto de Investigacion Agropecuaria de Panama with different kinds of crops and nematodes. He came to the University of Florida in 1982 to work toward his Doctor of Philosophy degree. He is a member of the Organization of Tropical American Nematologists and of the Society of Nematologists. He has been married to Olga Lydia Perez since 1980 and they have two daughters, Jesica Eileen and Elvia Lineth.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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August, 1986

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